SUSTAINABLE MANAGEMENT OF MANGO FRUIT ROT: BIOCONTROL OF *Diaporthe* BY *Streptomyces flaveus* RT1-1

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ABSTRACT

Mango is one of Vietnam's most important tropical fruit trees, valued not only for its appealing taste but also for its rich content of vitamins, minerals, and antioxidants that promote good health. Despite being easy to cultivate and adaptable to various soil conditions, mango trees are often susceptible to diseases that significantly impact their growth, development, yield, and fruit quality. Among these, fruit rot is a major concern, particularly stem-end rot, which can severely affect mangoes after harvest. Currently, disease management primarily relies on chemical fungicides, which pose serious risks to human health, contribute to environmental pollution, promote the emergence of drug-resistant fungi, and disrupt microbial ecosystems. In this study, a stem-end rot-causing fungus was isolated from mango fruits collected at Thanh Xuan Market, District 12, Ho Chi Minh City, Vietnam. The isolated fungus exhibited branched, septate hyphae and distinct α- and β-conidia. Pathogenicity was confirmed by re-inoculation onto fresh mango fruits, which developed typical rot symptoms. Molecular identification based on sequencing of an 820 bp rDNA fragment and phylogenetic analysis revealed that the isolate was closely related to *Diaporthe* strains. Accordingly, the isolate was named *Diaporthe* TX12 HCMC. The antifungal activity of *Streptomyces flaveus* RT1-1 against *Diaporthe* TX12 HCMC was evaluated through a co-culture assay on PDA. The result showed an average inhibition of 71.83 ± 1.82%, suggesting that extracellular metabolites produced by *S. flaveus* RT1-1 probably diffused into the agar medium and inhibited the fungal growth. These findings highlight the potential of *S. flaveus* RT1-1 as a biocontrol agent against *Diaporthe*-induced stem-end rot in mangoes, offering a promising alternative to synthetic fungicides for management of postharvest fruit rot.

Keywords: Sustainable agriculture, agricultural biotechnology, stem-end rot, mango, biocontrol, *Streptomyces flaveus*.

1. INTRODUCTION

Mango (*Mangifera indica* L.) is a vital tropical fruit in Vietnam, prized for both economic value and nutritional benefits, and is extensively grown for local use and export. Its high levels of vitamins, minerals, and antioxidants enhance its reputation as a health-promoting food. The species' ability to thrive in a range of soils and climatic conditions supports its widespread cultivation. Nonetheless, mango farming is increasingly hindered by the emergence of various plant diseases [1]. Fruit rot in mango, which can be caused by various pathogens, increasingly attributed to pathogens of the *Diaporthe* genus, has become a significant concern. This disease, commonly referred to as stem-end rot, typically begins at the fruit's pedicel. It starts as a dark brown to black spot and progresses into soft, brown decay accompanied by an unpleasant odor,

making the fruit unsuitable for consumption. Multiple *Diaporthe* species have been identified as contributors to stem-end rot in mangoes across various regions of the world [2-6]. However, there has been only one reported case of stem-end rot mango sold in Vietnam [7].

Diaporthe species are able to produce phomopsin-A (PHO-A). PHO-A is highly toxic to animals, especially ruminants like sheep, where it causes lupinosis, a serious liver disease marked by jaundice, weight loss, and even sudden death. It damages liver cells by binding to tubulin and disrupting microtubule function. Similar toxic effects, including liver tumors, have been seen in cattle and rats [8-9]. Due to its potency and potential to contaminate food, managing *Diaporthe* is essential to protect both animal and human health.

Pesticides have been recommended for management of the diseases, including fruit rot, in mangoes [1]. As a result, chemical fungicides have been widely used to manage the diseases. However, this strategy raises significant concerns, such as potential health hazards, environmental pollution, the emergence of fungicide-resistant pathogens, and harm to beneficial microbes. Permethrin, for instance, is a pesticide permitted for use in Vietnam [10] – its misuse has left its residues on mango fruits. In fact, Vietnamese mango products packaged in 5kg bags were found to contain residues of permethrin [11, 12]. Therefore, there is an urgent need for sustainable and eco-friendly alternatives to chemical fungicides.

Actinobacteria, particularly *Streptomyces* species, have gained significant attention for their potential as biocontrol agents due to their ability to produce antifungal metabolites. These naturally occurring soil bacteria can inhibit fungal pathogens by secreting bioactive compounds [13], making them promising candidates for environmentally safe disease management.

In this study, we aimed to isolate and identify the fungal pathogen responsible for mango fruit rot based on morphological and molecular approaches, and evaluate the biocontrol potential of *Streptomyces flaveus* RT1-1 for the fruit rot disease on mangoes.

2. MATERIALS AND METHODS

2.1. Materials

Both healthy and diseased mango fruits were obtained from Thanh Xuan Market in District 12, Ho Chi Minh City. The *S. flaveus* RT1-1 strain, originally isolated from soil, was maintained on starch-casein agar [14].

2.2. Methods

2.2.1. Isolation of stem-end rot causing agent

The fungal pathogen was isolated from diseased mangoes by surface sterilizing the fruit in 70% ethanol for 30 seconds, then 1% NaOCl for 2 minutes, followed by a rinse with sterile distilled water and drying on autoclaved paper. Tissue pieces (~ 1 cm^2) were excised from the boundary of healthy and infected areas and placed on 90 mm petri dishes containing potato dextrose agar (PDA) prepared as previously described [14], then incubated at 25 °C for 2 to 5 days. Developed mycelia were then checked regularly under a light microscope at 400× magnification. The fungi were sub-cultured repeatedly onto fresh petri dishes until pure isolates were obtained.

2.2.2. In vitro fungal infection

Three mango fruits weighing 200-250 g were prepared by washing under tap water,

surface-sterilizing with 70% alcohol, immersing in 1.5% NaClO for 3 minutes, and rinsing with sterile distilled water. A 9 mm diameter metal rod was sterilized with alcohol, heated on the flame until hot, cooled to room temperature, and used to create 0.5 cm deep wounds on the fruit. Agar plugs (9 mm wide, 5 mm thick) containing fungal mycelia were placed onto the wounds, and the fruits were incubated at 25 °C in nylon bags with moist sterile towels for 7 days. Following the development and spread of rot, the fungus was re-isolated on PDA, purified, and identified.

2.2.3. Fungal identification and phylogenetic analysis

Molecular identification of the fungal species was performed at the Molecular Diagnostics Department, Nam Khoa-Biotek Laboratory, Ho Chi Minh City (License No. 05505/SYT-GPHĐ). Genomic DNA was extracted and used as a template for PCR amplification targeting the ribosomal DNA (rDNA) region. The resulting PCR product was then sequenced and blasted against the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov). The rDNA fragments which were closely related to the PCR sequence were downloaded from the database and used for the phylogenetic analyses, using the MEGA12 software [15]. The ClustalW program within the software was used to perform multiple sequence alignment. Evolutionary history was inferred using the Neighbor-Joining method [16]. Evolutionary distances were calculated using the p-distance method [17], expressed as the number of base differences per site. Ambiguous positions were handled by applying the pairwise deletion option for each sequence pair. Bootstrapping was conducted with 1,000 replicates.

2.2.4. Antifungal activity testing

The antagonistic effect of the actinomycete strain against the pathogenic fungus was evaluated in vitro by co-culturing on PDA. The *S. flaveus* RT1-1 strain was sub-cultured onto the starch-casein agar, incubated at 30 °C for 3-5 days, then used to co-culture with the stem-end rot causing agent. The pathogenic fungus was inoculated at the center of the petri dish, and actinomycete strain was placed at the four corners, 3 cm from the center. Fungus cultured on a plate without actinomycete was used as a control. After incubating at 25 °C for 7 days, antifungal zones were examined, and the antagonistic effect I (Inhibition, %) of the actinomycete against fungal pathogen was determined using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{R1} - \text{R2}}{\text{R1}} \times 100$$

where R1 was the radius of the fungal colony in the control (cm), and R2 was that of the fungal colony in the presence of the actinomycete (cm) [18].

The antagonistic effect was determined in triplicate. The resulting inhibition values from these three replicates were used to compute the mean antagonistic effect and standard deviation, with the latter calculated using the STDEV.S() function in Microsoft Excel to reflect the variability within the experimental set.

3. RESULTS AND DISCUSSION

3.1. Isolation of mango fruit rot causing agent

Suspected stem-end rot mango fruits (Fig. 1a) were selected for fungal isolation. After the fungi grew on the medium containing the suspected diseased mango pieces, the fungal

strains were isolated and purified primarily based on the morphology of the aerial mycelia. One of the isolated strains, designated as TX12 HCMC, after subculture, appeared grayish-white in color, with brownish-yellow to black colloidal droplets on the surface. The fungus grew rapidly on PDA medium at 25 °C and formed either a fluffy or flat fungal colony on the culture medium (Fig. 1b).

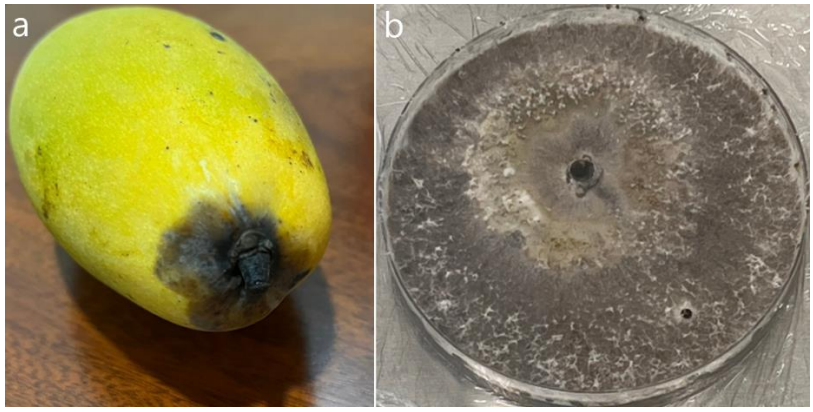

Figure 1. Stem-end rot mango fruit bought at the market (a) and morphology of the fungus isolated on PDA after 7 days of incubation (b)

3.2. Microscopic observation and in vitro fungal infection

Under the light microscope, the isolated fungal strain suspected of causing mango stem-end rot showed branched and septate hyphae (Fig. 2a). It possessed α-conidia, which were hyaline, aseptate, ellipsoidal to cylindrical, and rounded at both ends (Fig. 2b). It also had β-conidia, which were hyaline, filiform, and aseptate (Fig. 2c). After being artificially inoculated onto fresh mango fruits and incubated, rot symptoms were observed. At the artificially infected sites, dark brown to black spots appeared and spread, the tissues became soft, rot with an unpleasant odor (Fig. 2d).

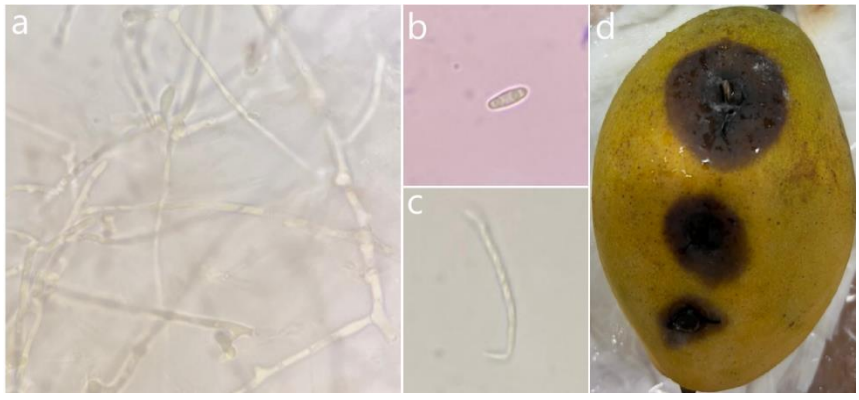

Figure 2. Morphology of the isolated fungus under the light microscope. (a) Fungal hyphae, (b) α-conidium, (c) β-conidium, (d) mango fruit infected with the isolated fungus artificially (9 mm diameter agar plates were removed)

The previous studies showed that different *Diaporthe* species can have different morphologies [19-21], however, because precise identification of plant pathogenic species is obligatory for quarantine and disease management, the identification of species beyond

morphology requires additional molecular sequence data [22, 23]. Thus, based on the fungal morphology, it was not accurate to identify the isolated strain.

Some previous studies also noted that not all *Diaporthe* species were capable of causing stem-end rot in mango, and their pathogenicity was also host-specific [4, 24]. In this study, the isolated strain caused infection in fresh mango fruits, so it was subsequently moved on to molecular identification.

3.3. Fungal identification and phylogenetic analysis

Following PCR amplification of the rDNA region from the fungal isolate's genome, the resulting product was sequenced, yielding an 820 bp fragment. A BLAST analysis against the NCBI database revealed high similarity to sequences encoding portions of the 18S, 5.8S, and 28S ribosomal RNA genes found in various *Diaporthe* strains. The BLAST search results showed that the PCR sequence shared over 95% identity with the top 100 rDNA sequences of strains within the genus *Diaporthe*. Figure 3 presents a phylogenetic tree constructed from 17 related rDNA sequences of comparable length. This tree indicates that the stem-end rot causing isolate, designated *Diaporthe* TX12 HCMC, was closely related to other *Diaporthe* strains.

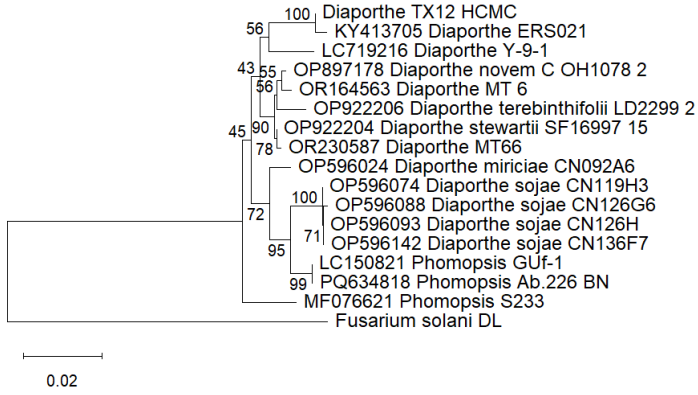

Figure 3. Phylogenetic relationships among the *Diaporthe/Phomopsis* strains. Numbers at the nodes are the bootstrapped values (in percent). The NCBI accession numbers are shown before the species names. *F. solani* DL sequence [25] was used as an outgroup.

The genus *Diaporthe*, first described by Nitschke in 1870 with D. eres as the type species, belongs to the family Diaporthaceae, order Diaporthales, within the class Sordariomycetes [26, 27]. *Diaporthe* is a genus of filamentous fungi with diverse ecological roles, acting as plant pathogens, endophytes, and saprobes. It has historically been associated with the asexual genus Phomopsis due to its ability to exhibit both sexual and asexual forms, which were traditionally named separately under the dual nomenclature system. To simplify fungal taxonomy, a unified naming system has been proposed, aiming to assign a single name to each species. However, transitioning to this system has proven difficult because both names, *Diaporthe* and *Phomopsis*, have been widely used in scientific and agricultural contexts. While *Phomopsis* is more familiar, especially in plant pathology, *Diaporthe* takes precedence under current nomenclature rules since it is the older name. Unless *Phomopsis* is formally conserved through an official process, all species should now be referred to as *Diaporthe* [28].

The rDNA sequence of the *Phomopsis* sp. strain UM 254 (Accession No. JX966551) used in this study was also utilized in a previous study, where a stem-end rot-causing strain, *Phomopsis* strain M2, was isolated from infected mango fruits and identified in Hanoi, Vietnam [7]. Compared to the strains shown in the phylogenetic tree (Fig. 3), this strain was

too distantly related and was therefore not included in the tree. Figure 4 shows a large difference in rDNA sequences of *Phomopsis* sp. UM 254 and *Diaporthe* TX12 HCMC.

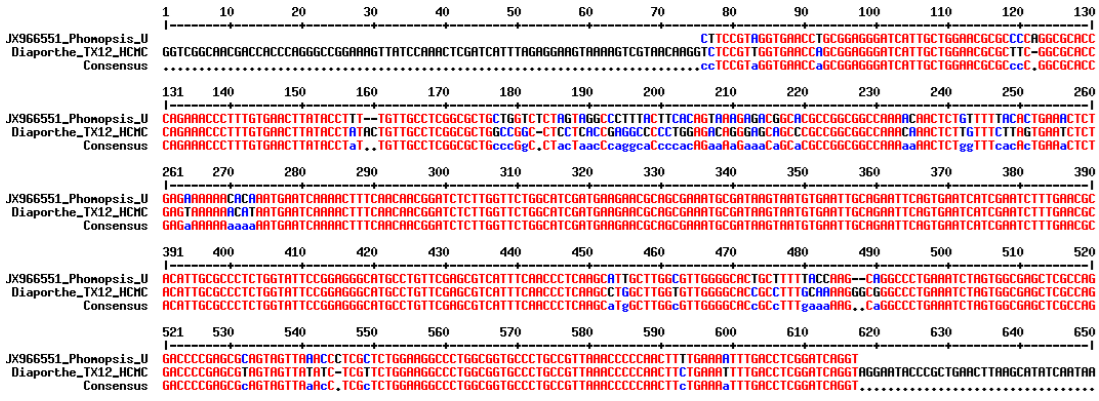

Figure 4. Difference in rDNA sequences of *Phomopsis* sp. UM 254 and *Diaporthe* TX12 HCMC. The PCR product's sequence was aligned with the rDNA sequence of *Phomopsis* sp. UM 254 obtained from the NCBI database, using an online program [29]. Numbers on top of the alignment show the nucleotide positions in the aligned sequences.

3.4. Antifungal activity

Diaporthe TX12 HCMC was co-cultured with *S. flaveus* RT1-1, a soil-derived isolate, and was found to be susceptible to its antagonistic effect. As shown in Figure 5a, fungal growth was suppressed in areas surrounding the hyphal mats of *S. flaveus*, while in the absence of the actinomycete, the fungus grew freely (Fig. 5b). This suggests that extracellularly inhibitory compounds released by *S. flaveus* had diffused into the agar and restricted the growth of *Diaporthe* TX12 HCMC.

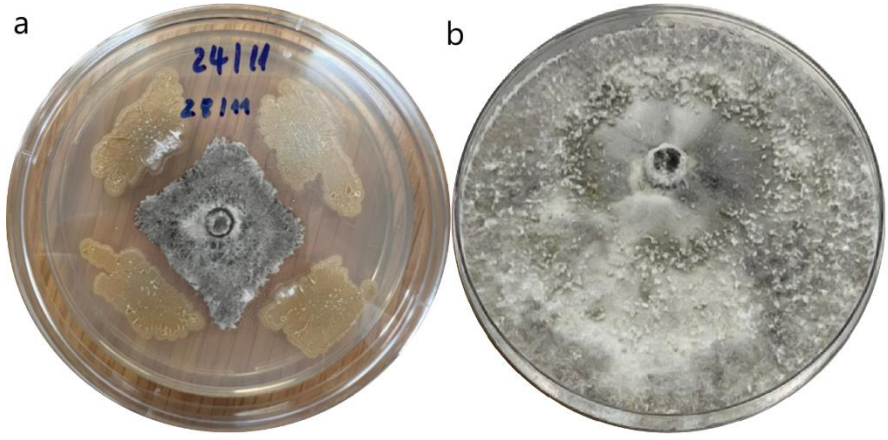

Figure 5. Co-culture of *Diaporthe* TX12 HCMC and *S. flaveus* RT1-1. (a) *Diaporthe* TX12 HCMC co-cultured with *S. flaveus* RT1-1, (b) Control.

The antagonistic effect of *S. flaveus* RT1-1 against *Diaporthe* TX12 HCMC is presented in Table 1. On average, the antagonistic effect was 71.83 ± 1.82%, indicating a substantially high level of activity.

Table 1. The antagonistic effect of *S. flaveus* RT1-1 against *Diaporthe* TX12 HCMC

Replicate	R1 (cm)	R2 (cm)	I (%)
1	4.2	1.25	70.24
2	4.2	1.2	71.43
3	4.2	1.1	73.81

S. flaveus RT1-1 has the ability to produce chitinases [30, 31]. Chitinases have the ability to cleave the chitin microfibrils inside the fungal cell wall and cause the fungal cell to decompose [32]. However, it cannot be ruled out that this actinomycete produces antifungal antibiotics. For example, *S. flaveus* strain A11 has been reported to produce antibiotic SW-B, which was capable of inhibiting the growth of various plant-pathogenic fungi [33]. Nevertheless, whether *S. flaveus* strain A11 can suppress the growth of the stem-end rot-causing fungus *Diaporthe* remains unknown. Therefore, further studies will aim to clarify the identity of the compounds involved in inhibiting the growth of fungi that cause the stem-end rot in mangoes.

4. CONCLUSION

Diaporthe TX12 HCMC is the first stem-end rot-causing fungus isolated from mango fruits in southern Vietnam. *S. flaveus* RT1-1 exhibited strong antagonistic activity against Diaporthe TX12 HCMC *in vitro*, with an average inhibition rate of $71.83 \pm 1.82\%$. These findings suggest that *S. flaveus* RT1-1 produced extracellular antifungal compounds capable of suppressing the growth of this postharvest pathogen. The results demonstrate the promising potential of *S. flaveus* RT1-1 as a biocontrol agent, providing a natural and sustainable alternative to synthetic fungicides for managing postharvest stem-end rot in mango. Further studies on the active compounds, their mechanisms of action, and field applications are warranted to advance the development of this biological control strategy.

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TÓM TẮT

QUẢN LÝ BỀN VỮNG BỆNH THỐI QUẢ XOÀI: KIỂM SOÁT SINH HỌC *Diaporthe* SỬ DỤNG *Streptomyces flaveus* RT1-1

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Xoài là một trong những loại cây ăn quả nhiệt đới quan trọng nhất của Việt Nam, không chỉ được ưa chuộng nhờ hương vị thơm ngon mà còn bởi hàm lượng cao các vitamin, khoáng chất và chất chống oxy hóa có lợi cho sức khỏe. Dù dễ trồng và thích nghi tốt với nhiều loại đất khác nhau, cây xoài vẫn thường mẫn cảm với các loại bệnh gây ảnh hưởng đáng kể đến quá trình sinh trưởng, phát triển, năng suất và chất lượng quả. Trong số các bệnh đó, bệnh thối quả là một mối lo ngại lớn, đặc biệt là thối cuống, gây ảnh hưởng nghiêm trọng lên chất lượng quả xoài trong giai đoạn sau thu hoạch. Hiện nay, việc quản lý bệnh thối quả chủ yếu dựa vào thuốc diệt nấm hóa học, song điều này tiềm ẩn nhiều rủi ro đối với sức khỏe con người, góp phần gây ô nhiễm môi trường, thúc đẩy sự xuất hiện của nấm kháng thuốc và làm rối loạn hệ sinh thái vi sinh vật. Trong nghiên cứu này, một chủng nấm gây bệnh thối cuống đã được phân lập từ quả xoài thu thập tại chợ Thạnh Xuân (Quận 12, Thành phố Hồ Chí Minh). Dưới kính hiển vi quang học, nấm có hệ sợi phân nhánh, có vách ngăn, có các bào tử α và β có hình dáng đặc trưng. Khả năng gây bệnh của nấm được xác minh thông qua lây nhiễm nhân tạo lên quả xoài tươi khỏe, làm xuất hiện các triệu chứng thối quả điển hình. Phân tích phát sinh loài dựa trên việc giải trình tự đoạn rDNA có kích thước 820 bp cho thấy chủng nấm phân lập được có mối quan hệ gần với các chủng *Diaporthe* đã biết. Do vậy, chủng nấm được định danh là *Diaporthe* TX12 HCMC. Khả năng đối kháng của *Streptomyces flaveus* RT1-1 đối với *Diaporthe* TX12 HCMC được đánh giá thông qua thí nghiệm đồng nuôi cấy trên môi trường PDA. Kết quả cho thấy khả năng ức chế trung bình đạt 71,83 ± 1,82%, chứng tỏ các hợp chất trao đổi chất ngoại bào do *S. flaveus* RT1-1 tiết ra có khả năng khuếch tán vào môi trường thạch và ức chế sự sinh trưởng của nấm. Các kết quả thu được cho thấy có thể sử dụng *S. flaveus* RT1-1 như một tác nhân sinh học tiềm năng trong việc kiểm soát bệnh thối quả xoài do *Diaporthe* gây ra, qua đó cung cấp một giải pháp thay thế thân thiện hơn cho thuốc diệt nấm hóa học trong quản lý bệnh thối quả xoài sau thu hoạch.

Từ khóa: Bệnh thối quả xoài, công nghệ sinh học nông nghiệp, kiểm soát sinh học, nông nghiệp bền vững, *Streptomyces flaveus*.